



Clean copy of amended specification

Please amend the fourth paragraph on page 3 as follows:

B2

Figure 4A and 4B presents a comparison of GUS expression resulting from transiently expressing the gus gene in plasmids p350096 (Fig. 4A) and pMuA0096 (Fig. 4B).

Please amend the second and last paragraphs on page 13 as follows:

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The yeast homolog to the CaMV 35S promoter produced a low level of transient expression when compared to the CaMV 35S promoter in transient expression experiments in corn callus (see Example 2). An increase in the level of expression was sought by changing some nucleotides thought to be important for the function of the CaMV 35S promoter. Three bases at positions 277, 278, and 279 of the yeast homolog (Figure 1) were changed from ACA to CGC by site directed mutagenesis using the QuikChange™ site-directed mutagenesis kit according to the manufacturer Stratagene (Figure 1). This new promoter called MuA (Figure 2) in plasmid pMuA0096 resulted in a similar level of transient expression in corn callus when compared to the CaMV 35S promoter (see Example 2, Figure 4A and 4B). The sequence of MuA (Figure 2) has 79.5% homology over 352 base overlap to the CaMV 35S promoter published by Gardner et al., 1981. The comparison of the MuA promoter with the CaMV 35 S promoter is shown in Figure 3.

DNA Delivery: A particle inflow gun (PIG) as described by Finer et al. (1992) and Vain et al. (1993) was used to deliver the DNA. In brief, 50 mg of tungsten particles (M10 from Sylvania Chemicals/Metals, Towanda, PA) were sterilized for 15 minutes in 95% ethanol in a 1.5 ml microfuge tube. Particles were rinsed 3 times in sterile distilled water by repeated vortexing, centrifugation and resuspension in 0.5 ml water. Particle suspensions were made fresh for each experiment. Plasmid DNA was coated onto the particles by mixing 25 ul of tungsten particle suspension (2.5 mg), 5 µl of DNA (5 ug), 25 ul of 2.5 M CaCl₂, and 10 ul of 100 mM spermidine (free base) . After allowing the particles to settle for a few minutes while on ice, 50 ul of supernatant was removed. Two ul of the remaining particle suspension was pipetted onto the center of the screen of a syringe filter unit. The syringe filter unit was reassembled and screwed into the LUER-LOK needle adaptor within the chamber. The target tissue in a petri plate was

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placed about 15 cm below the syringe filter unit. A vacuum of approximately 28 in Hg was applied and the particles were discharged when helium (80 psi) was released following activation of the solenoid by the timer relay.

Please amend the last paragraph on page 14 as follows:

B4

After bombardment, callus was incubated at 25°C in the dark for 16-24 h on the same medium used for bombardment. Then, transient gus expression was evaluated by incubating the tissue in 0.5 mg/ ml X-gluc (Gold Biotechnology, Inc. St. Louis, MO) in 0.1M sodium phosphate buffer pH 7.0 and 0.1% TRITON-X-100 at 37°C for 4-16 h after which the number and intensity of blue foci were evaluated under a stereo microscope at approximately 10x magnification. Tissue was transformed with either p350096, or pMuA0096. Results are shown in Figure 4A and 4B. It is seen that tissue transformed with p350096 (Fig. 4A) or pMuA0096 (Fig. 4B) had similar levels of transient expression. Tissue transformed with pY0096 was found to have lower levels of transient expression.

Please amend the last paragraph on page 15 as follows:

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Immature embryos of Stine elite inbred 963 were aseptically removed from kernels of plants grown in a grow room (15h photoperiod, 28° day and 25° night). Embryos were harvested 10 to 11 days after pollination when they were between 1 mm and 2 mm in length and then placed in 2 ml of LSinf medium (Table 2) in an Eppendorf tube. The mixture was then stirred with a vortex mixer (VORTEX GENIE 2) at full speed for 5 seconds, the LSinf removed, replaced with fresh medium and then stirred again. All medium was then removed from the tube using a Pasteur pipette. Bacteria were collected with a platinum loop (enough to coat the wire of the loop) and thoroughly suspended in 1 ml of Lsinf-AS medium (Table 2) using a Pasteur pipette. The bacterial suspension was then introduced into the tube containing the embryos and the mixture stirred with a vortex mixer at full speed for 30 seconds. After this the embryos were allowed to stand for five minutes and were then transferred to the surface of LSAS medium (Table 2)

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solidified with agar, care being taken to remove any accompanying liquid. Embryos were immediately oriented so that the scutellar surface was uppermost.